Importance of Direct Spray and Spray Residue Contact for Infection of *Trichoplusia ni* Larvae by Field Applications of *Beauveria bassiana*

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ABSTRACT Commercial formulations and unformulated conidia of *Beauveria bassiana* strain GHA were applied to field-grown plants and artificially infested with *Trichoplusia ni* (Hübner) larvae to compare the relative insecticidal activity resulting from direct spray contact with insecticidal activity due to contact with dry spray residue. In general, applications to cabbage, *Brassica oleracea* L., resulted in nearly equal mortalities when comparing insects exposed to direct spray contact with those exposed by spray residue, suggesting a potential benefit by improving formulations to extend residual activity. For applications to beans, *Phaseolus vulgaris* L., direct spray contact provided significant insect mortality, but mortality due to residual contact was generally not different than the untreated control. In contrast to the differences observed for larvae exposed in the field, larvae exposed in laboratory bioassays to leaf disks collected from the same treated cabbage and bean plants (residual contact exposure) resulted in nearly identical mortalities. Field applications of *Beauveria* showed rapid loss of activity, expressed as a loss of conidia viability and loss of insecticidal activity during the first 8 h after application. Evidence of significant mortality by residual contact and the rapid loss of insecticidal activity with field exposure support additional research to improve formulations to extend the residual activity of fungal biopesticides.

KEY WORDS *Beauveria bassiana*, *Trichoplusia ni*, direct spray contact, residual activity

Microbial pesticides have the undesirable characteristic of providing inconsistent pest control when applied to field environments. This variability can be the result of many factors, including the crop being treated, plant developmental stage, stage of the target insect, weather, application technique, formulation and spray coverage. Biopesticides made with *Beauveria bassiana* have great commercial potential due to a wide host range, but they must contact the target pest to initiate infection. This contact may result from direct spray contact or be picked up from dried spray residue by insects as they forage on treated plant tissue. A better understanding of the relative importance of each of these contact mechanisms will help direct formulation research to improve efficacy of fungal biopesticide applications.

Our laboratory has worked to extend the residual activity of biological insecticides by improving the ability of formulations to resist environmental degradation of the active agent after application in the field, and specifically by preventing degradation by exposure to sunlight (McGuire et al. 2000). Extending the residual activity is a viable strategy for agents that require contact with spray residue for activity such as bacteria and baculoviruses that initiate infection after ingestion by a susceptible pest. A spray-drying technique has been used to successfully encapsulate bacterial (Tamez-Guerra et al. 2000b) and baculovirus (Tamez-Guerra et al. 2000a, Behle et al. 2003) agents in a lignin matrix, which absorbs UV energy and protects the microbe from degradation.

For microbial agents with contact activity, such as *Beauveria bassiana*, the mode of contact may be an important factor affecting insecticidal activity. Fungi initiate infection through the exoskeleton, and they are less effective if ingested (Jeffs et al. 1997). Without additional evidence, one may consider direct contact by the spray application to be more important for inducing infection compared with insects contacting dried spray residue and therefore conduct formulation research to maximize direct contact. This further assumes that some of the resident insects are missed by the spray application and that spray coverage (and efficacy) can be improved by reducing the number of missed insects. If residual contact is shown to be effective for infection, then research efforts may be better spent on increasing the probability of the pest contacting an active spray residue, such as improving residual activity by preventing degradation by sunlight. It is known that conidia exposed to sunlight quickly lose insecticidal activity (Fargues et al. 1996, Morley-Davies et al. 1996, Inglis et al. 1997); therefore, an alternative logical strategy could favor developing formulations to protect the fungus from light exposure

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to improve efficacy of the agent by extending the residual activity after application. This logic assumes that the residual contact of the target pest with treated substrate provides successful transfer of the fungus to initiate the infection process and that the "protective" formulation will not hinder this process. Even without specific evidence supporting the role of residual contact for insecticidal efficacy, formulations research was initiated to protect conidia viability from degradation by sunlight exposure. The spray-dried lignin formulation developed for *Bacillus thuringiensis* and baculovirus has been adapted for encapsulation of conidia of *B. bassiana* for control of *Lygus* spp. in field margins before they infest cotton (Leland and Behle 2005). Also, a modified soybean oil (SoyScreen) has been developed as an oil-based sunscreen (Compton and Laszlo 2000) and tested as an ingredient in oil-based formulations for *B. bassiana* (R.W.B., unpublished data).

It is likely that the relative impacts due to direct spray contact and residual contact are unique to each pest control situation. The cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), is a recognized pest of many crops, and vegetables, such as soybean, cotton, cabbage, cauliflower, broccoli, tomatoes, peas, and leaf crops, and it is susceptible to infection by *B. bassiana.* Often, these crops can tolerate some leaf-feeding damage, typically caused by this caterpillar, without suffering economic loss; thus, *T. ni* is a good candidate for pest control by slower acting microbial-based pesticides. However, the crop itself may impact levels of pest control resulting from spray and residual contact. Fernandez et al. (2001) demonstrated that spray contact with *B. bassiana* caused 76% mortality of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), larvae compared with 34% mortality for contact with residue on treated leaves and 77% mortality for combined contact. While developing bioassay techniques, Lui et al. (2003) demonstrated that immersing *Lygus lineolaris* (Palisot de Beauvois) in fungal suspensions (direct spray exposure) was the most effective inoculation method compared with insect exposure to treated broccoli, *Brassica oleracea botrytis* (L.), florets or bean pods (residual exposure). House flies, *Musca domestica* L., and stable flies, *Stomoxys calcitrans* (L.), were susceptible to *B. bassiana* by residual contact with treated plywood (Watson et al. 1995) (no direct contact reported). Red imported fire ant, *Solenopsis invicta* Buren, was susceptible to *B. bassiana* by direct contact with dry applications or sprays of suspended conidia, but not by residual contact when the conidia were mixed with the soil substrate (Stimac et al. 1993a,b). For leaf feeding caterpillars, it is likely that residual contact provides a significant contribution to insect mortality such that extending residual activity of biopesticide applications will benefit pest control efficacy, so long as the infection mechanisms are not disrupted. The cabbage looper was selected as the model lepidopteran plant pest for comparing the relative impact of spray contact and residue contact when applied to different crops to demonstrate similarities, differences, or both among unique pest/crop control situations. Cabbage and beans were selected as model crops because they have been used as exposure medium for laboratory bioassays (R.W.B., unpublished data) and they represent crops from different plant families, crucifer and legume, respectively.

For these experiments, it was hypothesized that residual contact is important for infection of cabbage looper larvae by *B. bassiana* such that extending residual activity can improve efficacy of fungal-based biopesticides. The goal was to control the exposure of larvae to direct spray contact and dry residue contact in a way to measure the resulting mortality for each exposure. *B. bassiana* was selected as the candidate agent because of the depth of information already published about this organism, and strain GHA was selected because it is currently available in two commercial formulations. To support the general hypothesis, a wide range of exposure conditions (numerous formulations, multiple treatment dates, and alternative crops) were used. This article reports the results of field applications in which beans and cabbage plants were artificially infested with laboratory-reared neonate cabbage looper before and after applications of commercial formulations and unformulated conidia of *B. bassiana.* Larval applications and collections were timed to separate insect mortality due to direct spray contact from insect mortality due to contact with dried spray residue. Additional information was collected to document the loss of conidia viability in support of observations on insecticidal activity of field applications.

Materials and Methods

***Beauveria* Strain and Formulations.** *B. bassiana* strain GHA conidia were produced using proprietary commercial methods and provided as a technical powder (1.49×10^{11} conidia per g), BotaniGuard ES (2.3×10^{10} conidia per g), and Botaniguard WP (4.4×10^{10} conidia per g) by Emerald Bio (formerly Mycotech Corporation, Butte, MT; now Emerald Bioagriculture Corporation, Lansing, MI). Technical powder containing conidia was used alone as unformulated. Thus, four treatments (unformulated conidia, two commercial formulations, and an untreated control) were compared in the following experiment.

Field Design. This experiment was established as a split-plot design over two crops conduced at the USDA–ARS National Center for Agricultural Utilization Research, Peoria, IL. Treatments were applied on four dates, two applied to cabbage ('Bravo' F1 hybrid, Harris Seeds, Rochester, NY) and two applied to green bean ('Savannah', Harris Seeds). Cabbage seeds were germinated in individual peat pots in a greenhouse on 30 March 2004 before transplanting to field plots on 28 April. When transplanted, cabbage plants were spaced 60 cm apart in 15 rows that were 18 m in length and 120 cm apart. Beans were hand seeded (18 June 2004) in 15 rows that were 18 m in length and 120 cm apart. A uniform block of four rows was selected from within a planting for each application date. Applications were

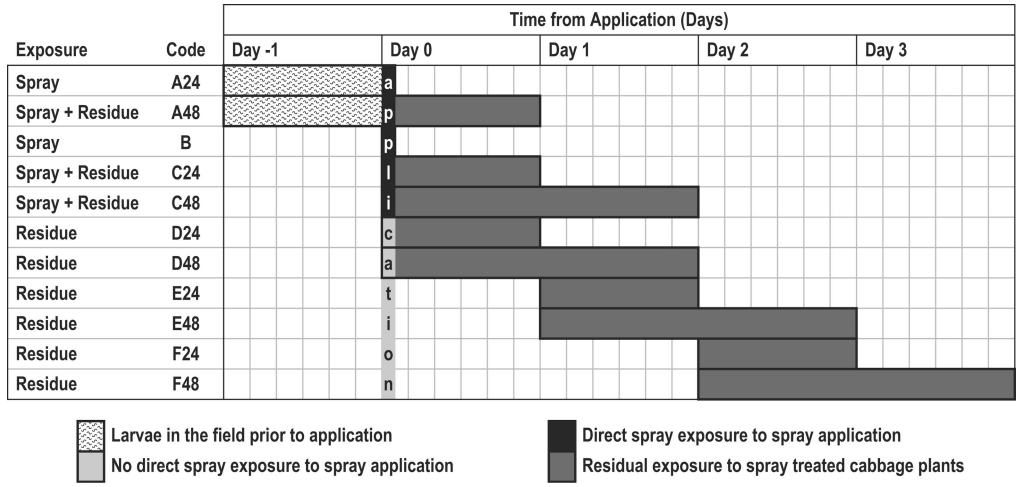

Fig. 1. Gantt chart for codes representing the exposure times for *T. ni* larvae artificially infested on field-grown cabbage and bean plants that were treated with *B. bassiana* at time 0, day 0.

made between 6:30 and 7:30 a.m. local time to cabbage on 15 and 29 June and to bean plants on 26 July and 7 September (cabbage I, cabbage II, beans I, and beans II, respectively). All *Beauveria* treatments were applied at 2.47×10^{13} conidia per ha (1×10^{13} conidia per acre), the label rate for commercial products. Sprays were applied with a CO_2-charged backpack sprayer at 248 kPa (36 psi) through three TXVS 6 Conejet nozzles (Spraying Systems Co., Wheaton, IL) directed at the row, one nozzle over the row and one nozzle on each side of the row. Nozzles were arranged in a triangle with 35 cm between the center and side nozzles and 65 cm between the side nozzles. The spray angle of the side nozzles was centered ≈35 cm below the central nozzle, which was directed down.

All four applications followed the same procedure. Each treatment was applied to a row of plants, 18 m in length. Each of the four rows was divided into six sections, and each 3-m section was infested with excessive numbers (>300 per section of row sprinkled over the top of the plants) of laboratory-grown cabbage looper neonates at different times. The section codes, exposure definition, and infestation timings relative to spray application are represented graphically by the Gantt chart (Fig. 1). Section A was infested 24 h before application and collected just after application (A24, direct spray) and again the next day (A48, direct spray + residual contact). Sections B and C were infested just before application, ≈6:00 a.m. Larvae from section B were collected just after application ≈8:30 a.m. (B, direct spray contact) and larvae from C were collected 24 and 48 h later (C24 and C48, direct spray + residual contact). Sections D, E, and F were infested ≈2, 26, and 50 h after application, respectively, and larvae were collected at 24 and 48 h after being placed in the field (D24, D48, E24 E48, F24, and F48, residual contact). Only live larvae were collected by using a fine artist brush to transfer insects from plants to individual 29.6 ml (1-oz.) cups containing wheat germ diet modified from Gardiner (1985) (Behle et al. 2000). For each row section × collection time, 60 larvae were collected, filling cups of two 30-well trays, and each tray of 30 larvae was considered a subsample to estimate mortality. These insects were incubated at 28°C for 5 or 6 d before evaluating for the percentage of mortality at 7 d after initial exposure to the treated plants. Dead larvae that did not feed from the diet were considered to have been killed by the transfer and were not counted.

Ambient weather conditions in the field were recorded during the experiment with a data logger (LI-COR 1400, LI-COR, Lincoln, NE) weather station. Data include hourly temperature (1400-102 Air Temperature Sensor 2, LI-COR), rain accumulation (tip bucket, 1400-106, LI-COR), and light (pyranometer, LI-200SA, LI-COR). Additionally, relative humidity was recorded by an alternative weather station (Davis Instruments Corp., Hayward, CA) located ≈200 m from the plots and maintained by National Center for Agricultural Utilization Research greenhouse personnel.

Conidia Viability. Samples were collected from each formulation mix before application to determine the concentration of viable conidia based on techniques originally described by Luz and Fargues (1997). Three samples (1.7 ml) were transferred into each of three shaker-flasks, each containing 50 ml of yeast extract broth, and incubated for 14 h at 28°C and 280 rpm. Yeast extract broth consisted of 2 g of yeast extract (Difco, Detroit, MI) and 2 g of sucrose per liter of water. The numbers of germinated (germ tube > spore radius) and nongerminated (no germ tube) conidia were determined for 100 conidia per flask observed microscopically. The concentration of conidia in each flask was determined using a hemacytometer (Bright-line, Hausser Scientific, Horsham, PA). The concentrations of viable conidia were determined by multiplying the conidia concentration by the percentage of germinated conidia for each shaker-

flask sample. Each shaker flask was considered a replicate for viability.

Leaf Imprints for Fungus Viability. Four leaf disks (38 mm in diameter) were cut from selected leaves within each of the field treatments, avoiding the midrib of the cabbage leaves, or from the center of the selected leaflet of bean leaves. Leaf disks were briefly pressed, top-side down, onto to the surface of *Beauveria*-selective modified SDA media (Doberski and Tribe 1980), one leaf disk per agar plate, to transfer fungus from the leaf surface to the surface of the media. The selective media were prepared by combining and autoclaving 65 g of Sabouraud Dextrose Agar (Sigma-Aldrich, St. Louis, MO), 0.01 g of crystal violet (Sigma-Aldrich), 0.5 g of chloramphenicol (Sigma-Aldrich), and 1,000 ml of deionized water. After autoclaving, 0.25 g of cyclohexamide (Sigma-Aldrich) was added, and media were poured into 90- by 15-mm petri dishes (Falcon, Becton Dickson Labware, Franklin Lakes, NJ). After the dishes were inoculated with the leaf disks, they were incubated in an incubator (Innova 4230 incubator, New Brunswick Scientific, Edison, NJ) at 25°C. After 7 d, the dishes were evaluated for number of colony-forming units (CFUs) by using a binocular dissecting microscope (Wild M8, Leica, Heerbrugg, Switzerland). Because few colonies grew on the agar for the untreated control samples, all fungal colonies were counted except those that with obviously different morphological characteristics from the typical white *Beauveria* colonies. Each leaf imprint was considered a replication of the treatment.

Laboratory Bioassay for Residual Insecticidal Activity. In addition to the field-exposed larvae, 10 leaves were randomly collected throughout the canopy from treated plants in each treatment at 2, 8, 26, and 50 h after application to assess residual activity of the fungus. One leaf disk (38 mm in diameter) was cut from a leaf, and leaf disks were placed individually (top-side up) in 50- by 9-mm petri dishes (Falcon, Becton Dickson Labware). Then, 10 neonates were placed in each dish for a 24-h exposure to the treated leaf tissue. After exposure, six larvae per dish (two trays of 30 larvae each per treatment) were transferred to individual diet cups and incubated in the dark at 28°C for 6 d before assessing mortality. Each tray was considered a replicate for determining mortality for each treatment.

Comparing Direct Spray Contact with Spray Residue Contact. The infestation and sampling procedure provided specific comparisons for exposure to direct spray contact (direct application of treatments) with exposure to dried spray residues on treated plants. Thus, spray contact is represented directly by codes A24 and B, and by subtracting the effect of corresponding residual contact as represented by C24-D24 and C48-D48. Likewise, mortality due to residual contact is represented directly by codes D24 and D48, and indirectly by subtracting mortality due to corresponding spray contact as represented by A48–A24, C24-B, and C48-B. Sections E and F were intended to demonstrate extended residual activity of applications.

Data were analyzed using SAS System for Windows version 8 (SAS Institute, Cary, NC). The experimental design was a split block design where the treatment applications represent replications. Replications for each application × treatment include two trays (30 larvae per tray) collected for each row-section code, two trays (30 larvae) from 10 leaf disk samples for each treatment × sample time for laboratory bioassay, four leaf disk samples for leaf imprints (for each sample time), and three shaker-flasks for each *Beauveria* treatment for determining conidia germination. For comparing contact with residual activity, mortality data for field-collected larvae were analyzed for significant interactions among the main effects (crop treated, section code for evaluation, and *Beauveria* treatment). When interactions were not significant, the mortality data for the three *Beauveria* treatments were considered to be three replications for comparing effects of exposure. Data were analyzed by analysis of variance (ANOVA) using PROC GLM, and treatment means were separated by Tukey's studentized range test as the option selected for the LSmeans statement. For paired comparisons of direct spray mortality with residue contact mortality, means were separated using the least significant difference (LSD) option for the means statement. For CFUs from leaf imprints, the CFU data were transformed, log(CFU + 1), before conducting ANOVA.

Results

Conidia Viability. Evaluating treatments for conidia concentration and germination percentage was intended to demonstrate similarity of applications to the field plots. Treatments of *Beauveria* did not differ in the concentration of total viable conidia that were applied to the plants for three of the four applications. The BotaniGard WP applied to beans II had significantly ($F_{2,\ 6} = 5.63$; $P = 0.0419$) more viable spores than the unformulated treatment. This difference in total viable conidia was primarily a reflection of a higher conidia count for the BotaniGard WP formulation, beans II application (Table 1). When combining the data for the four applications, there was no significant ($P > 0.05$) formulation × crop interaction, although there were interactions for application date × formulation for the number of conidia ($F_{6,\ 24} = 3.80$; $P = 0.0048$) and total number of viable conidia ($F_{6,\ 24} = 3.09$; $P = 0.0219$). These interactions were a reflection of the high spore count for BotaniGard WP, beans II application.

Leaf Imprints for Fungus Viability. Leaf imprints on selective agar indicated that all applications of *Beauveria* significantly increased the presence of the pathogen in the treated plots. Imprints from leaf samples from plants treated with *Beauveria* collected 2, 26, and 50 h after application had significantly more CFUs compared with leaf samples from untreated plants (Table 2). Also, CFUs declined with additional exposure in the field, and this decline was greatest during the first 24 h of exposure. Over the three *Beauveria* treatments, imprints from leaf disks collected 2, 26,

Table 1. Germination percentage and number of *B. bassiana* conidia per milliliter (× 10^4) for formulations applied to field-grown cabbage and bean plants

Formulation	Cabbage I		Cabbage II		Beans I		Beans II	
	%	No.	%	No.	%	No.	%	No.
BotaniGuard ES	89.0	246	88.7	166	84.3	246	92.0a	234b
BotaniGuard WP	81.7	200	81.0	146	81.3	220	82.7b	417a
Conidia powder	89.0	186	82.0	146	87.0	214	88.0ab	206c
$F_{2, 6}$	1.87	1.43	3.78	0.19	1.07	0.37	10.76	8,012
P	0.2339	0.3115	0.0865	0.8337	0.4008	0.7023	0.0104	0.0196

Means in a column followed by the same letter are not significantly different (Tukey's studentized range test, $P < 0.05$).

and 50 h after application averaged 1,105, 531, and 352 CFUs per leaf disk, respectively. Note that the 2-h average is a low estimate because it is calculated without a value for the beans II application, for which CFUs were too many to count and would have been >2,000 CFUs per leaf disk.

Laboratory Bioassay for Residual Insecticidal Activity. Residual insecticidal activity of the *Beauveria* formulations was evaluated based on leaf-disk samples collected from plants and returned to the laboratory for evaluation (Tables 3 and 4 for cabbage and beans, respectively). Low levels of mortality (<13% mortality) were observed for larvae exposed to leaf samples collected from the control (no *Beauveria* treatment) plots. Comparing between the two plants, larvae exposed to leaf disks from beans treated with *Beauveria* had significantly higher mortality than larvae exposed to leaf disks from treated cabbage collected 26 h after treatment ($F = 8.21$; df = 1, 24; $P = 0.0085$), but they were not different for samples collected 2, 8, or 50 h after treatment ($P > 0.05$) (Fig. 2). When applied to cabbage plants, *Beauveria* applications caused significantly greater larval mortality than the no *Beauveria* control only for leaf samples collected 2 h after application (Table 3). When applied to beans, BotaniGard ES caused significantly greater insect mortality compared with larvae exposed to untreated leaf disks for each of the four samples collected up to 50 h after application (Table 4). Unformulated conidia caused significant mortality for larvae collected up to 26 h after application, but mortality for the BotaniGard WP treatment was greater than the control mortality for only the 2-h sample.

Among the three *Beauveria* applications averaged for all four applications, BotaniGard ES provided the highest average mortality (42%) followed by unformulated conidia (33%), and BotaniGard WP (23%) (Tukey's honestly significant difference [HSD] minimum difference, 6.6%). Comparing among sample times, leaf samples from the three *Beauveria* treatments that were collected 2, 8, 26, and 50 h after application provided 67, 31, 21, and 11% mortality, respectively (Tukey's HSD minimum difference, 11.4%). These leaves were exposed to an average total photosynthetic radiation energy (400–700-nm wavelengths) of 33, 297, 434, and 787 kJ m^{-2} of light energy, respectively for 2-, 8-, 26-, and 50-h exposures, respectively.

Comparing Direct Spray Contact with Spray Residue Contact. Averaged mortality for larvae collected for different infestation codes from untreated control plants ranged from 1.1 to 15.1% mortality for cabbage and 0.8–14.7% mortality for beans, and mortality among the different codes were not significantly different ($P > 0.05$) when analyzed separately for each crop. For each of the four application dates ($n = 11$ codes), no *Beauveria* control mortality averaged (± SD) 8.7 ± 8.1, 5.9 ± 4.4, 2.9 ± 2.2, and 9.8 ± 9.4% for cabbage I, cabbage II, bean I, and bean II applications, respectively. Larvae exposed in the field to *Beauveria*-treated cabbage generally had higher mortalities than larvae exposed to *Beauveria*-treated bean plants (Table 5). ANOVA of the full model indicated no significant ($P < 0.05$) interactions for *Beauveria* treatments with crop, evaluation code, or both; thus, the data for the three *Beauveria* treatments were considered replications for comparing direct contact with residual contact mortalities. Considering only larvae exposed to *Beauveria*-treated plants, residual contact only for 48 h always had numerically greater mortality than the corresponding larvae exposed for 24 h. Unfortunately, insecticidal activity was rapidly lost after application as mortalities for larvae exposed one (E24 and E48 codes) and 2 d (F24 and F48 codes) after application were not significantly different ($P < 0.05$) from re-

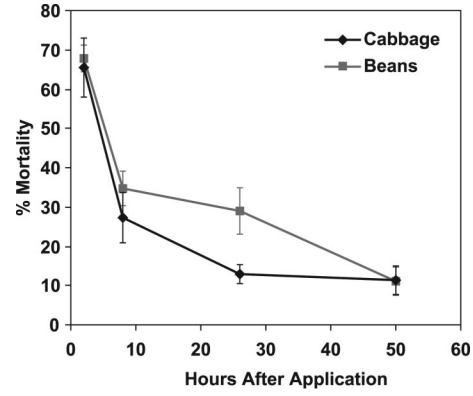

Fig. 2. Insecticidal activity (average percentage of mortality) for neonate *T. ni* exposed to cabbage or bean leaf disk collected 2, 8, 26, and 50 h after application from plants treated with *B. bassiana* formulated as Conidia Powder, BotaniGard ES, and BotaniGard WP treatments, average of two experiments for each crop.

Table 2. CFUs from leaf imprints collected after field applications of *B. bassiana* formulations, average of four leaf disk imprints

Formulation	Cabbage I			Cabbage II			Beans I			Beans II		
	2 h	26 h	50 h	2 h	26 h	50 h	2 h	26 h	50 h	2 h	26 h	50 h
BotaniGard ES	1,858a	66a	46	2,098a	228a	314a	2,167a	1,312a	1,242a	—[a]	1,584a	1,220a
BotaniGard WP	1,292a	146a	100	592b	406a	229ab	1,829a	751a	960a	—	1,032a	456b
Conidia powder	1,213a	19ab	28	325b	328a	75b	1,580a	958a	818a	—	1,482a	443b
Control	8b	0b	0	2c	1b	3c	20b	2c	0c	42	58b	0c
$F_{3,12}$	217.63	8.83	2.22	66.75	43.04	44.11	115.37	112.09	281.04		34.67	247.06
P	<0.0001	0.0023	0.1385	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		<0.0001	<0.0001

Raw data were transformed, log (CFU + 1), for ANOVA, and means followed by the same letter were not significantly different (Tukey's studentized range test, $P < 0.05$).

[a] Colonies were too numerous to count.

Table 3. Dry spray residue contact percentage of mortality of neonate *T. ni* exposed for 24 h to leaf disks from field-grown cabbage plants cut at various times after being treated with formulations of *B. bassiana*

Formulation	2 h	8 h	26 h	50 h
BotaniGard ES	80.6 ± 11.3a	40.3 ± 12.5	18.6 ± 5.1	10.0 ± 3.6
BotaniGard WP	48.9 ± 10.1a	12.7 ± 4.8	8.7 ± 3.0	6.8 ± 3.0
Conidia powder	67.0 ± 14.9a	29.3 ± 11.8	11.8 ± 3.2	17.5 ± 9.9
Control	1.7 ± 1.0b	2.6 ± 2.6	8.5 ± 3.4	4.2 ± 2.5
$F_{7,8}$	10.46	3.46	1.56	1.06
P	0.0011	0.0510	0.2505	0.4029

Means ± SE in a column followed by the same letter were not significantly different (Tukey's studentized range test, $P < 0.05$).

spective mortalities for larvae collected from no-*Beauveria* control plants. Larvae placed in the field 24 h before the biopesticide application (codes A24 and A48) had lower average mortality than those placed on plants just before the application (codes B, C24, and C48).

Table 6 presents specific comparisons of mortality due to direct spray contact with mortality from contact with spray residue. For these comparisons, only mortality data from the three *Beauveria* treatments were used for analysis. Data from untreated plots was omitted because these data are intended to verify that observed mortality was a result of *Beauveria* treatments, but otherwise these data do not contribute information for comparing *Beauveria* mortality. Three treatments of *Beauveria* were not significantly ($P > 0.05$) different, and treatments did not have significant ($P > 0.05$) interactions with other main effect variables, including crop, application date, or evaluation code. There was, however, a significant interaction between crop and contact (spray versus residue) main effects ($F = 20.14$; df = 1, 80; $P < 0.0001$). Thus, the data for the two crops comparing mortality for direct spray contact with mortality due to residue contact were analyzed separately. The paired comparisons (Table 6) indicate about equal impact for direct spray and residual contact on larval mortality when exposed to treatments applied to cabbage, but significantly greater activity for direct spray contact when applied to beans. These comparisons demonstrate that insecticidal activities for spray and residual contacts differ between these two crops when larvae were exposed in the field.

Table 4. Dry spray residue contact percentage of mortality of neonate *T. ni* exposed for 24 h to leaf disks from field-grown bean plants cut at various times after being treated with formulations of *B. bassiana*

Formulation	2 h	8 h	26 h	50 h
BotaniGard ES	71.0 ± 7.5a	49.2 ± 3.6a	47.5 ± 8.6a	21.0 ± 8.7a
BotaniGard WP	68.7 ± 4.6	21.7 ± 4.0bc	9.1 ± 2.7bc	4.3 ± 1.7ab
Conidia powder	63.6 ± 6.1a	33.3 ± 7.2ab	30.4 ± 7.3ab	8.0 ± 3.2ab
Control	12.2 ± 3.3b	6.8 ± 2.8c	0.0 ± 0.0c	0.8 ± 0.8b
$F_{7,8}$	24.96	14.59	13.60	3.48
P	<0.0001	0.0003	0.0004	0.0505

Means ± SE in a column followed by the same letter were not significantly different (Tukey's studentized range test, $P < 0.05$).

Table 5. Mean percentage of mortality of *T. ni* larvae collected from artificially infested field-grown cabbage and bean plants that were treated with applications of three *B. bassiana* formulations (conidia powder, BotaniGard ES, and BotaniGard WP)

Exposure	Code	Cabbage	Beans
Spray	A24	13.0 ± 2.0d	3.2 ± 1.4c
Spray + residue	A48	34.7 ± 8.9bcd	5.5 ± 1.4c
Spray	B	40.6 ± 2.0bcd	24.8 ± 5.3b
Spray + residue	C24	82.2 ± 7.4a	38.1 ± 10.6ab
Spray + residue	C48	83.9 ± 2.3a	44.8 ± 7.9a
Residue	D24	47.1 ± 10.6bc	5.5 ± 1.6c
Residue	D48	57.1 ± 5.5ab	8.7 ± 2.3c
Residue	E24	11.6 ± 5.1d	3.1 ± 2.2c
Residue	E48	23.8 ± 6.4bcd	5.7 ± 2.6c
Residue	F24	12.0 ± 4.0d	1.6 ± 0.6c
Residue	F48	14.3 ± 4.8cd	4.2 ± 1.0c

A, infested 24 h before application; B and C, infested just before application; D, infested 2 h after application; E, infested 24 h after application; F, infested 48 h after application; 24, larvae collected 24 h after infestation; and 48, larvae collected 48 h after infestation. Codes are depicted graphically in the Gantt chart, Fig. 1.

Means in a column followed by the same letter were not significantly different (Tukey's studentized range test, $P < 0.05$).

Environmental conditions were relatively consistent among the four applications considering range of dates. For the 5 d after each application, temperatures averaged 22.5, 23.6, 22.5, and 20.3°C for cabbage I, cabbage II, bean I, and bean II applications, respectively. Three rain events of 2, 15, and 3 mm recorded 15 June, 3 July, and 29 July, respectively, did not greatly impact these experiments because of the small amount (<5 mm) or length of time (>3 d) after treatment application. For the first 24 h after application, when the highest insect mortality was recorded, relative humidity averaged (mean ± SD) 79 ± 9, 69 ± 21, 73 ± 20, and 76 ± 10 for cabbage I, cabbage II, bean I, and bean II applications, respectively. During the 5 d after applications, the lowest humidity recorded was 42%, and the average humidity for the each of the four applications was 75 ± 14, 73 ± 18, 76 ± 19, and 70 ± 16%.

Table 6. Exposure codes and mortality of *T. ni* larvae comparing direct spray exposure with residue contact to cabbage and bean plants treated with three formulations of *B. bassiana* (conidia powder, BotaniGard ES, and BotaniGard WP) based on data reported in Table 5

Direct spray contact			Residue contact		
Treatment code	% mortality		Treatment code	% mortality	
	Cabbage	Bean		Cabbage	Bean
A24 =	13.0	4.2	A48–A24 =	21.7	1.4
B =	40.6	24.8	C24–B =	41.6	13.3
C24–D24 =	35.0	32.6a	D24 =	47.1	5.5b
C48–D48 =	25.5B	36.1a	D48 =	57.1A	8.7b
Avg	30.2B	22.6a		38.0A	9.5b

A, infested 24 h before application; B and C, infested just before application; D, infested 2 h after application; 24, larvae collected 24 h after infestation; and 48, larvae collected 48 h after infestation. Codes are depicted graphically in the Gantt chart, Fig. 1.

Means in a row followed by the same uppercase letter (cabbage) or lowercase letter (bean) are not significantly different (LSD, $P < 0.05$).

Discussion

This research demonstrates the impact of direct spray contact and residual spray contact on insecticidal activity of *Beauveria* applications in the field. It is important to test *Beauveria* under a range of conditions to provide knowledge of the potential for this control agent to be effective against a range of pests, which occur under equally wide environmental conditions. The design of this study was directed at this purpose. Although a single target insect was used, the four application dates and two crops were intended to provide variability inherent among field applications. By exposing the applications to these conditions, a more robust test was performed to compare the impact of direct spray contact with residual contact as it relates to insect mortality. Among these evaluations, the young insects exposed to both direct spray contact and contact with dried spray residue exhibited the highest mortality. Spray contact provided more consistent efficacy for the applications made to these two crops. Yet, the results demonstrated that contact with spray residue provides significant additional mortality of the target pest, especially when applied to cabbage plants. Longer contact with residual tended to increase larval mortality (48-h exposure compared with 24-h exposure), adding additional evidence that residual contact adds to the efficacy of these applications even as efficacy declines rapidly with additional field exposure. The results reported here contrast with the low mortality of *L. lineolaris* when exposed to *Beauveria*-treated broccoli, which was ≈80 times less compared with that of insects exposed directly to sprays (Leland and Behle 2005). Other research also has demonstrated a wide range of results for comparing spray contact with residual contact of insects exposed to fungal biopesticides. When studying the interaction of *Beauveria* applications with several species of beneficial arthropods used for pest control in greenhouses, mortalities of beneficial arthropods varied widely and ranged from 4.9 to 60.0% when exposed to wet spray residue and from 4.3 to 46.3% when exposed to dried spray residue (Ludwig and Oetting 2001).

In contrast to cabbage, *Beauveria* applications to bean plants did not provide the same level of mortality for *T. ni* when insects were exposed in the field. Insects applied to beans and exposed to direct spray contact expressed about one-half the mortality as insects applied to cabbage when sprayed. Mortality differences between insects exposed to different host plants were not unexpected. Kouassi et al. (2003) demonstrated higher mortality of *L. lineolaris* exposed to *Beauveria* applications on lettuce compared with those exposed to applications on celery. Inyang et al. (1998) found that larvae of beetle *Phaedon cochleariae* (F.) acquired more conidia when fed on oilseed rape than on cabbage or turnip, but they suggested fungistatic compounds of the rape plants interfered with the infection process. Poprawski and Jones (2001) reported that fungal inhibitors produced by cotton plants reduced germination of fungal conidia and conferred protection to *Bemisia argentifolii* Bellows & Perring, in con-

trast to uninhibited conidial germination and higher insect mortality when tested on melons. Beyond the impact of plant chemistry, observation reported here of different mortalities for insects exposed on treated cabbage and bean plants may have resulted from differences in plant architecture. Leaf architecture of cabbage is well suited for spray coverage of both the top and bottom of most leaves by the three-nozzle configuration used to apply the biopesticide treatments, whereas outer bean leaves tended to shield the interior of the canopy and underside of the leaves. CFUs from leaf imprints suggest differences in spray deposition on the upper surfaces between cabbage and bean plants, with beans having greater concentrations of *Beauveria* on the upper surface of the bean leaves. Unfortunately, the undersurfaces (where many larvae were collected from bean plants) were not tested by the imprinting technique to verify a lower concentration of *Beauveria.* The low insect mortalities observed for field applications to beans remains somewhat perplexing when considering that mortalities were similar or higher for beans when insects were exposed to treated leaf disks (residue contact only) in the laboratory (Fig. 2). Mortality of larvae exposed to dried spray residue in the field averaged 38.0% for cabbage plants and 9.5% for bean plants (for three *Beauveria* applications) compared with averages (over four sample times) of 29.3 and 35.7% mortality for larvae exposed to treated cabbage and bean leaf disks in the laboratory. Apparently, specific field conditions (such as weather conditions during exposure to treated leaf tissue) that limited insecticidal efficacy of the *Beauveria* treatments against insects exposed in field bean plots were nullified when insects were exposed to treated leaf tissue under laboratory conditions. Higher concentrations of spray residues on the upper surface of bean leaf disks, placed right-side up in the petri dishes, may partially account for this observation by preferentially exposing laboratory larvae to this part of the leaf, whereas larvae in the field fed predominantly from the undersurface of bean leaves away from concentrated spray residue.

The residual activities of these *Beauveria* applications were short as demonstrated by the rapid decrease of insect mortality for larvae exposed to treated plants just a few hours postapplication. Both field-collected larvae and laboratory assays of field-collected leaf tissue showed that most of the activity was lost during the first day after application in the field. This loss of activity likely resulted from exposure to sunlight, but other factors, such as desiccation of conidia after hydration by the application water, should be considered as contributing to reduced viability of conidia. Additionally, ambient weather conditions are known to play a role in the infection process, with high relative humidity providing the greatest benefit for infection (Fargues and Luz 2000). Records of weather conditions for each of the evaluation periods do not suggest abnormal or widely variable conditions that correlate to the loss of insecticidal activity after the first day of exposure.

Regarding formulations, Wraight and Ramos (2002) demonstrated better efficacy of the BotaniGard ES compared with Botanigard WP for control of Colorado potato beetle and attributed this benefit to resistance to wash-off by rain. Rain events were not considered to significantly impact our experiments, and data presented here would suggest only a slight preference for the ES over the WP formulation based on efficacy. LSD only separated these two formulations for field-exposed larvae collected for code C48, in which the ES formulation had significantly greater mortality than all other *Beauveria* treatments, when averaged over the four experiments. Mortality also was greater for larvae exposed to bean leaf disks treated with the ES formulation collected 8 and 26 h after application, compared with insects exposed to leaf disks from plants treated with the WP formulation.

The differences in the results observed for these two crops illustrate the need to research specific control situations in an effort to optimize the potential for biological control agents. Because residual contact provides significant insect mortality in some situations, the potential exists to improve biopesticide efficacy by developing proper formulations to extend the residual activity for field applications. The mortality of insects exposed to spray residues on cabbage demonstrated the potential for benefit of extending the residual activity beyond the few hours observed in our experiments. Previous research demonstrated that lignin encapsulation had potential benefits expressed as slower loss of conidia viability when exposed to simulated solar radiation but that these formulations were less pathogenic to *L. lineolaris* compared with noncoated conidia (Leland and Behle 2005). However, insecticidal efficacy for applications to beans could be improved by developing a suitable formulation to maximize plant coverage by sprays and facilitate transfer of the fungus from the plant surface to the target insect. Additional research is needed to identify suitable formulations to provide these benefits without reducing spore viability during processing or by interfering with the normal infection process.

References Cited

Behle, R. W., M. R. McGuire, and P. Tamez-Guerra. 2000. Effect of light energy on alkali-released virions from *Anagrapha falcifera* nucleopolyhedrovirus. J. Invertebr. Pathol. 76: 120–126.

Behle, R. W., P. Tamez-Guerra, and M. R. McGuire. 2003. Field activity and storage stability of *Anagrapha falcifera* nucleopolyhedrovirus (*Af*MNPV) in spray-dried lignin-based formulations. J. Econ. Entomol. 96: 1066–1075.

Compton, D. L., and J. A. Laszlo. 2000. Sunscreens from vegetable oil and plant phenols. US Patent no. 6,346,236.

Doberski, J. W., and H. T. Tribe. 1980. Isolation of entomogenous fungi from elm bark and soil with reference to ecology of *Beauveria bassiana* and *Metarhizium anisopliae* [*Scolytus scolytus* F., the main vector of Dutch elm disease]. Trans. Br. Mycol. Soc. 74: 95–100.

Fargues, J., and C. Luz. 2000. Effects of fluctuating moisture and temperature regimes on the infection potential of

Beauveria bassiana for *Rhodnius prolixus.* J. Invertebr. Pathol. 75: 202–211.

Fargues, J., M. S. Gottel, N. Smits, A. Ouedraogo, C. Vidal, L. A. Lacey, J. C. Lomar, and M. Rougier. 1996. Variability in susceptibility to simulated sunlight of conidia among isolates of entomopathogenic Hyphomycetes. Mycopathologia 135: 171–181.

Fernandez, S., E. Groden, J. D. Vandenberg, and M. J. Furlong. 2001. The effect of mode of exposure to *Beauveria bassiana* on conidia acquisition and host mortality of Colorado potato beetle, *Leptinotarsa decemlineata.* J. Invertebr. Pathol. 77: 217–226.

Gardiner, B.O.C. 1985. *Mamestra brassicae,* pp. 381–385. *In* P. Singh and R. F. Moore [eds.], Handbook of insect rearing, volume II. Elsevier Publishers Co., New York.

Inglis, G. D., D. L. Johnson, and M. S. Goettel. 1997. Effects of temperature and sunlight on mycosis (*Beauveria bassiana*) (Hyphomycetes: Sympodulosporae) of grasshoppers under field conditions. Environ. Entomol. 26: 400–409.

Inyang, E. N., T. M. Butt, L. Ibrahim, S. J. Clark, B. J. Pye, A. Beckett, and S. Archer. 1998. The effect of plant growth and topography on the acquisition of conidia of the insect pathogen *Metarhizium anisopliae* by larvae of *Phaedon cochleariae.* Mycol. Res. 102: 1365–1374.

Jeffs, B. L., M. G. Feng, J. E. Falkowski, and G. G. Khachatourians. 1997. Infections of the migratory grasshopper (Orthoptera: Acrididae) by ingestion of the entomopathogenic fungus *Beauveria bassiana.* J. Econ. Entomol. 90: 383–390.

Kouassi, M., D. Coderre, and S. I. Todorova. 2003. Effect of plant type on persistence of *Beauveria bassiana.* Biocontrol Sci. Technol. 13: 415–427.

Leland, J. E., and R. W. Behle. 2005. Coating *Beauveria bassiana* with lignin for protection from solar radiation and effects on pathogenicity to *Lygus lineolaris* (Heteroptera: Miridae). Biocontrol Sci. Technol. 15: 309–320.

Liu, H., M. Skinner, and B. L. Parker. 2003. Bioassay method for assessing the virulence of *Beauveria bassiana* against tarnished plant bug, *Lygus lineolaris* (Hem., Miridae). J. Appl. Entomol. 127: 299–304.

Ludwig, S. W., and R. D. Oetting. 2001. Susceptibility of natural enemies to infection by *Beauveria bassiana* and impact of insecticides on *Ipheseius degenerans* (Acari: Phytoseiidae). J. Agric. Urban Entomol. 18: 169–178.

Luz, C., and J. Fargues. 1997. Temperature and moisture requirements for conidial germination of an isolate of *Beauveria bassiana,* pathogenic to *Rhodnius prolixus.* Mycopathologia 138: 117–125.

McGuire, M. R., R. W. Behle, H. N. Goebel, and T. C. Fry. 2000. Calibration of a sunlight simulator for determining solar stability of *Bacillus thuringiensis* and *Anagrapha falcifera* nuclear polyhedrovirus. Environ. Entomol. 29: 1070–1074.

Morley-Davies, J., D. Moore, and C. Prior. 1996. Screening of *Metarhizium* and *Beauveria* spp. conidia with exposure to simulated sunlight and a range of temperatures. Mycol. Res. 100: 31–38.

Poprawski, T. J., and W. J. Jones. 2001. Host plant effects on activity of the mitosporic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* against two populations of *Bemisia* whiteflies (Homoptera: Aleyrodidae). Mycopathologia 115: 11–20.

Stimac, J. L., R. M. Pereira, S. B. Alves, and L. A. Wood. 1993a. *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycetes) applied to laboratory colonies of *Solenopsis invicta* Buren (Hymenoptera: Formicidae) in soil. J. Econ. Entomol. 86: 348–352.

Stimac, J. L., R. M. Pereira, S. B. Alves, and L. A. Wood. 1993b. Mortality in laboratory colonies of *Solenopsis invicta* (Hymenoptera: Formicidae) treated with *Beauveria bassiana* (Deuteromycetes). J. Econ. Entomol. 86: 1083–1087.

Tamez-Guerra, P., M. R. McGuire, R. W. Behle, J. J. Hamm, R. H. Sumner, and B. S. Shasha. 2000a. Sunlight persistence and rainfastness of spray-dried formulations of baculoviruses isolated from *Anagrapha falcifera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 93: 210–218.

Tamez-Guerra, P., M. R. McGuire, R. W. Behle, B. S. Shasha, and L. J. Galan-Wong. 2000b. Assessment of microencapsulated formulations for improved residual activity of *Bacillus thuringiensis.* J. Econ. Entomol. 93: 219–225.

Watson, D. W., C. J. Geden, S. J. Long, and D. A. Rutz. 1995. Efficacy of *Beauveria bassiana* for controlling the house fly and stable fly (Diptera: Muscidae). Biol. Control 5: 405–411.

Wraight, S. P., and M. E. Ramos. 2002. Application parameters affecting field efficacy of *Beauveria bassiana* foliar treatments against Colorado potato beetle *Leptinotarsa decemlineata.* Biol. Control 23: 164–178.

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